

Abstract

The present work studies the effect of metal ions on the structure, dynamics and interactions of the protein ShhN, the N-terminal signaling domain of Sonic Hedgehog. To accomplish this task, molecular structures of ShhN proteins were analyzed with a set of computational methods, revealing new features of ShhN proteins.

The results suggest that, ShhN is an enzyme with a zinc catalytic center that is regulated by the binding of the calcium ions. Explicitly, the binding of the second calcium ion involves a conformational change that is accompanied by a significant perturbation of the putative catalytic center, possibly affecting substrate stabilization. The dragging of E127 towards the calcium center implies the pulling of H135 with it and the disruption of the hydrogen bond between G128 and H141. Besides, the distance between residues E177 and H135 increases and therefore, the well-defined position of the catalytic water molecule is lost destabilizing the zinc environment. Electrostatic potential differences among calcium states suggest the possible binding of nonpolar substrates. One of the predictions is that ShhN autodegrades tuning its own concentration gradient. This possibility does not rule out, of course, the existence of other mechanisms that govern ShhN concentration gradient. The novel switching mechanism proposed could have many implications in the biological function of HhN proteins, but these are not well understood and require further research.

Both ShhN monomers and dimers show a flexibility pattern that strongly depends on the number of calcium ions. Specially, calcium binding loops reflect this behavior. The Cardin-Weintraub motif located within the N-terminal of ShhN proteins together with buried hydrophobic residues at the interface lead to a stable complex that enhances ShhN dimerization. The lower degree of conservation of I48 in vertebrate homologs might indicate that this is a hot spot residue with an important role in ShhN oligomerization. The presence of the calcium ions at the dimeric interface can promote ShhN-proteoglycan interactions providing a large positively charged region which is the ideal scenario for the binding of these molecules.

Taken all together, a multimerization model where different levels of interaction can control the way that ShhN multimers form is proposed. However, this model has yet to be tested.